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Single-Dose and Steady-State Pharmacokinetics of Hypericin and Pseudohypericin

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Single-dose and steady-state pharmacokinetics of antivirally acting hypericin (H) and pseudohypericin (PH) were studied in 13 healthy volunteers by administration of St. John's Wort extract LI 160, a plantal antidepressant. Oral administration of 250, 750, and 1,500 μg of H and 526, 1,578, and 3,156 μg of PH resulted in median peak levels in plasma (C_{max}) of 1.3, 7.2, and 16.6 μg/liter for H and 3.4, 12.1, and 29.7 μg/liter for PH, respectively. The C_{max} and the area under the curve values for the lowest dose were disproportionally lower than those for the higher doses. A lag time of 1.9 h for H was remarkably longer than the 0.4-h lag time for PH. Median half-lives for absorption, distribution, and elimination were 0.6, 6.0, and 43.1 h after 750 μg of H and 1.3, 1.4, and 24.8 h after 1,578 μg of PH, respectively. Fourteen-day treatment with 250 μg of H and 526 μg of PH three times a day resulted in median steady-state trough levels of 7.9 μg/liter for H and 4.8 μg/liter for PH after 7 and 4 days, respectively; the corresponding C^{ss}_{max} levels were 8.8 and 8.5 μg/liter, respectively. Kinetic parameters after intravenous administration of *Hypericum* extract (115 and 38 μg for H and PH, respectively) in two subjects corresponded to those estimated after an oral dosage. Both H and PH were initially distributed into a central volume of 4.2 and 5.0 liter, respectively. The mean distribution volumes at steady state were 19.7 liters for H and 39.3 liters for PH, and the mean total clearance rates were 9.2 ml/min for H and 43.3 ml/min for PH. The systemic availability of H and PH from LI 160 was roughly estimated to be 14 and 21%, respectively. Treatment with *Hypericum* extract, even in high doses, was well tolerated.

The naphthodianthrone hypericin (H) and its 2′-hydroxymethyl derivative, pseudohypericin (PH), (Fig. 1) are synthesized by plants of the *Hypericum* species, such as *Hypericum perforatum* L. (St. John's Wort) (4). H and PH chemically resemble anthraquinone laxatives but do not share their laxative effects. Extracts of St. John's Wort have long been used in traditional herbal medicine for wound healing and as an antidepressant (13). Recently, great interest has focused on the antiviral activity of H and PH (17, 20), which affects enveloped viruses such as herpes simplex virus, cytomegalovirus, and human immunodeficiency virus type 1 (1, 3, 7, 17, 18). Furthermore, the use of H as an inactivator of retroviruses in blood products has been suggested (16), and it may also be useful as an anticancer drug (9). The antiviral potency of H and PH was shown to require activation by visible light in a dose-dependent manner (5, 14). On the other hand, illumination with sunlight may cause so-called hypericism, a phototoxic reaction observed in grazing animals after excessive ingestion of St. John's Wort (2, 12).

Although antidepressive therapy with H and PH has been well established, little is known about their pharmacokinetics and their therapeutic indices even though preliminary studies on the antiviral efficiency in humans have been conducted. This study was designed to assess single-dose and steady-state pharmacokinetics of H and PH in healthy volunteers after oral administration of a solidified *Hypericum* extract. A high-performance liquid chromatography (HPLC) procedure that allows for sensitive monitoring of levels in plasma was used. Intravenous pharmacokinetics was investigated in two subjects so that an estimate of the systemic availability of H and PH from the solidified *Hypericum* extract was possible. Moreover, tolerance of high-dose treatment was evaluated.

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MATERIALS AND METHODS

Subjects. Fifteen male volunteers (aged 25 to 30 years) took part in the study. The median (range) body weight was 75 (62 to 82) kg, and the median height (range) was 180 (162 to 190) cm. Subjects were determined to be healthy on the basis of medical history, pre- and posttrial physical examination, electrocardiography, urine analysis, and routine tests of biochemistry, hematology, and virology (for hepatitis A and B and human immunodeficiency virus). All subjects gave written and informed consent, and the protocol was approved by the local ethics committee.

Study protocol. The study consisted of two parts. (i) In part I, a double-blind, random-order trial, subjects (n = 12) received three single oral doses of either 300, 900, or 1,800 mg of *Hypericum* extract (one, three, or six coated tablets of verum were supplemented by five, three, or zero placebos, respectively) at 8 a.m. Each dosage was separated by at least 10 days. Blood samples (10 ml) were collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 25, 48, 72, and 120 h after drug intake. Urine was collected in four intervals up to the next morning. Blinding controlled for the documentation of possible side effects.

(ii) Part II, a steady-state study, (n = 13) followed after a 4-week washout interval; 300 mg of *Hypericum* extract was taken every 5 h (8 a.m., 1 p.m., and 6 p.m.) for 14 days (days 1 to 14). On days 1 and 14 (pharmacokinetic sampling days), 10-ml blood samples were obtained prior to and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 h after the first two drug intakes and 1 and 2 h after the third dosing. From days 2 to 12, the volunteers reported daily (except on weekends) to the laboratory to receive their drug supply and to give a blood sample before the morning dose. On Saturdays and Sundays no blood samples were taken, and the volunteers received their medication with detailed instructions in advance on Friday. During washout, blood samples were collected at 24, 48, and 72 h and then every second day until 7 to 9 days after the last dose. Twenty-four-hour urine samples were collected in four fractions on days 1 and 14. Blood was withdrawn into syringes containing EDTA. After centrifugation the plasma was immediately stored at −80°C in polypropylene tubes. All samples were protected from light during handling.

Upon entry into part I and during part II of the study, urine samples were collected on random days for 24 h to screen for cannabinoids, amphetamines, morphines, benzodiazepines, barbiturates, and cocaine. Subjects avoided any drugs 2 weeks before and during the study. Smoking and alcohol were forbidden; urinary cotinine was checked several times to exclude nicotine use. No xanthine-containing beverages or food were taken from 10 p.m. the night before until 24 h after the administration of single doses. During part II of the study, one cup of

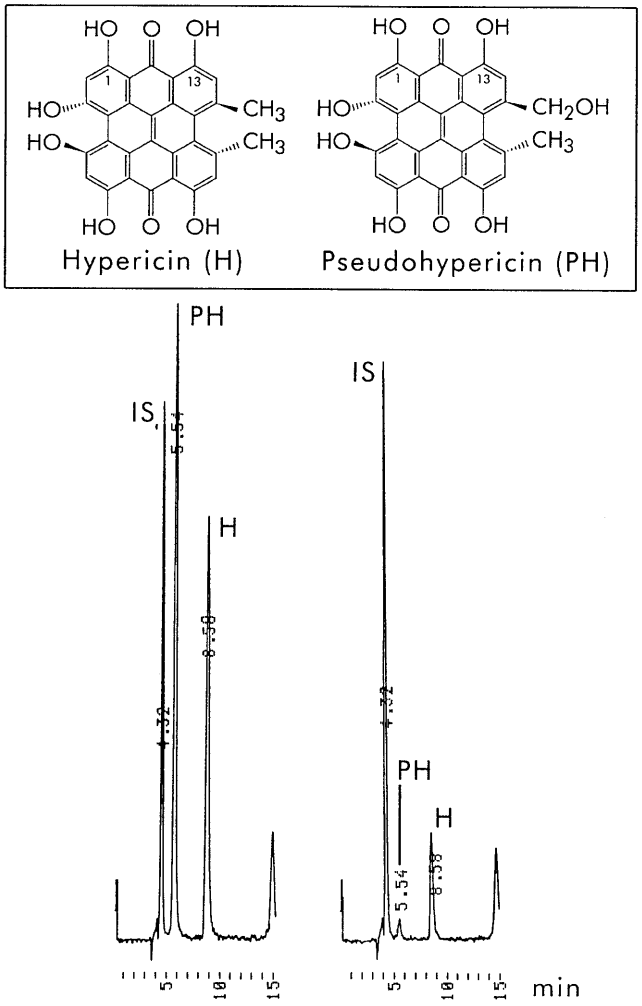

FIG. 1. HPLC chromatograms of extracts from human plasma samples obtained 4 (left panel) and 72 (right panel) h after oral administration of 1,800 mg of *Hypericum* extract containing 1,500 μg of H and 3,156 μg of PH. IS, internal standard dansylamide; PH, 34.9 and 0.32 μg/liter, respectively; H, 26.5 and 2.5 μg/liter, respectively.

coffee per day was allowed. Before each single-dose administration and prior to the morning doses on days 1 and 14 of steady-state treatment, the volunteers fasted overnight. The test drug was always taken before meals and followed by 2 h of fasting. Tolerability of the drug was recorded regularly by standardized questions.

Supplemental studies. In one volunteer (participant of part I), the kinetics of chemically pure H (750 μg in 10 ml of 10% [wt/vol] ethanol) administered orally was studied. The kinetics of H and PH administered intravenously, was studied in two subjects. *Hypericum* extract diluted in glucose 5%, corresponding to 115 μg of H and 38 μg of PH, was infused at a constant rate of 1 ml/min for 12 min. Blood samples were taken prior to and 5, 7, 9, 11.5, 15, 20, 30, 60, and 90 min and 2, 3, 4, 5, 6, 8, 24, and 36 h after the start of infusion.

Medication. Lichtwer Pharma (Berlin, Germany) supplied coated tablets (LI 160/PK, batch no. 0753) containing 300 mg of St. John's Wort extract. By the specific HPLC method described below, the content of each coated tablet was 250 μg of H and 526 μg of PH, with coefficients of variation (CVs) of 3.3 and 4.8% ($n = 10$), respectively. Chemically pure H, derived from a plantal extract by chromatographic purification, was dissolved in 10% ethanol to achieve a concentration of 750 μg/10 ml. St. John's Wort extract for parenteral application (Hyperforat, CH.-B.:539) was from Klein Arzneipflanzenforschung (Zell-Hammersbach/Schwarzwald, Germany). One-milliliter ampoules of 50 μg of total hypericins (H and PH) per ml contained specifically (by HPLC) 31.9 μg of H per ml and 10.6 μg of PH per ml. Six ampoules were diluted to 20 ml with 5% glucose, 12 ml of which was given to the subjects.

Analysis of H and PH. A previously reported HPLC assay (19) was adapted for extraction and determination of H and PH in one assay run. The HPLC was improved to increase the sensitivity approximately 200-fold. Ethanolic stock solutions of H (100 mg/liter), PH (100 mg/liter), and dansylamide (625 μg/liter, chosen as an internal standard) were stored in amber vials at −80°C. Pooled normal donor plasma was spiked with stock solutions of H and PH to give calibration standards ranging from 0.25 to 20 μg/liter and to prepare quality control samples of 0.5, 2, 5, 10, 20, and 40 μg/liter (stored at −80°C).

Reagents. Crystalline H (molecular mass, 504 Da) was obtained from Roth (Karlsruhe, Germany). PH (520 Da) was chromatographically purified from St. John's Wort (Analyticon, Berlin, Germany). The molar extinction coefficient of H (ε of 44,000 mM^{-1} cm^{-1} at 590 nm) was used for calibration of PH stock solutions (10). Dansylamide and β-glucuronidase (type X-A, 40×10^6 U/g) were obtained from Sigma-Aldrich (Deisenhofen, Germany). Methanol, ethyl acetate, dimethyl sulfoxide, and acetonitrile (all from Merck, Darmstadt, Germany) and tetrahydrofurane (Sigma-Aldrich) were of HPLC grade; 2-butoxyethanol (Sigma, St. Louis, Mo.) and all other chemicals were of analytical grade.

Extraction and HPLC. In a stoppered glass tube, 250 μl of plasma was spiked with 10 μl of an internal standard stock solution and mixed with 200 μl of dimethyl sulfoxide and then with 75 μl of acetonitrile–2-butoxyethanol (75:25 [vol/vol]). After each addition the mixture was vortexed for 15 s. The mixture was extracted with 2 ml of ethyl acetate, and after gentle shaking in a water bath at 37°C for 15 min and immediate centrifugation at $7,000 \times g$ for 5 min, the organic supernatant was removed. The pellet was reextracted by the same procedure. Both extracts were collected and dried in a vacuum centrifuge (Speed Vac; Savant Instruments). The dried extracts were redissolved in 100 μl of acetonitrile, and 20 to 50 μl was injected into the HPLC system.

The reversed-phase column (250 by 4 mm) was filled with LiChrospher 60 RP select B (Merck) and was kept at 60°C. The mobile phase (flow rate, 0.8 ml/min) was composed of methanol-tetrahydrofurane-buffer (45:30:25 [vol/vol/vol]). The buffer consisted of 0.1 M Na_2PO_4 (pH 4.0). A Shimadzu (Kyoto, Japan) model RF 551 spectrofluorometric detector, with the excitation wavelength set to 315 nm and emission detected at 590 nm, was used (Fig. 1).

Urine samples. The extraction and HPLC processes for urine samples were the same as those for plasma samples. To assess the presence of glucuronides, urine samples were diluted 1:1 (vol/vol) in a glass vial with β-glucuronidase–arylsulfatase (16,000 U/ml, dissolved in 0.5 M phosphate buffer [pH 6.8]) and incubated at 37°C for 12 h.

Assay precision. The lower quantification limit was 0.2 μg/liter (400 pmol/liter) when 250-μl plasma samples were used. The mean values for extraction efficiencies (CVs in parentheses) were 78.6% (8.9%) for H and 63.2% (11.3%) for PH. To control day-to-day variability, control samples were extracted at the same time in each run. The interassay precision (CV) values were 22.2, 13.7, 13.1, 12.2, 4.5, and 14.2% for H and 28.9, 14.4, 13.9, 8.8, 6.1, and 14.9% for PH for the corresponding concentrations of 0.5 ($n = 10$), 2 ($n = 8$), 5 ($n = 36$), 10 ($n = 7$), 20 ($n = 9$), and 40 ($n = 18$) μg/liter. The values for intraassay precision tested by repetitive analysis of the control samples containing 5 μg (each) of H and PH per liter were 6.5% for H and 12.8% for PH ($n = 10$). The results reported for the study samples are the means of duplicate measurements. The short-term storage of whole blood samples at either 4°C, room temperature, or 37°C prior to analysis did not change the H and PH concentrations. This aspect was tested with spiked whole blood up to 4 h. Furthermore, no relevant binding to or penetration into blood cells was detected; analysis of spiked whole blood revealed that all H and PH was present in the plasma fraction after centrifugation at 4°C, 37°C, and room temperature.

Pharmacokinetics. The highest concentration in plasma (C_{max}) and the corresponding sampling time (t_{max}) were read directly from the concentration in plasma-time data for each subject. The peak concentration in plasma at steady state (C^{SS}_{max}) was derived from the concentration in plasma-time profile for day 14; trough levels (C^{SS}_{min}) were calculated as the means of the morning concentrations in plasma (prior to dosing) measured from day 7 to day 14. The peak-trough fluctuation of levels in plasma during steady state was calculated as $(C^{SS}_{max} - C^{SS}_{min}) \cdot 100/C^{SS}_{max}$ (8). The lag time of absorption (t_{lag}) was estimated from the concentration in plasma-time curves (22). The concentration in plasma-time curves were fitted by least-squares nonlinear regression analysis according to the following macro constant function with first-order input and biexponential output: $C(t) = C_1 \cdot e^{-\alpha \cdot (t - t_{lag})} + C_2 \cdot e^{-\beta \cdot (t - t_{lag})} - (C_1 + C_2) \cdot e^{-k_a \cdot (t - t_{lag})}$, where k_a, α, and β are the rate constants for absorption, distribution, and elimination, respectively. For intravenous administration, a three-exponential disposition function with two distribution rate constants (α_1 and α_2) and one elimination rate constant gave the best fit to the data. For nonlinear regression analysis, library models 14 and 19 of PCNONLIN, version 4.2, were used (11). Iterative reweighting was calculated as $1/f^2$, where f is the predicted concentration. The pharmacokinetic models considered were the one-, two-, and the three-exponent disposition models. The main criterion for assessment of goodness of fit was random (alternating) distribution of residuals around the zero line. Furthermore, the appropriateness of the one-, two-, and three-exponent disposition models was compared by the use of the Akaike and the Schwarz criteria as calculated by PCNONLIN (11). According to these criteria, for oral treatment and for intravenous administration the two-compartment model and the three-compartment model, respectively, were superior to the other disposition models ($P < 0.001$, t test).

The AUC from time zero to infinity ($AUC_{0-\infty}$) was calculated from the fitted parameters, for the repeated dosage regimen the AUC corresponds to one

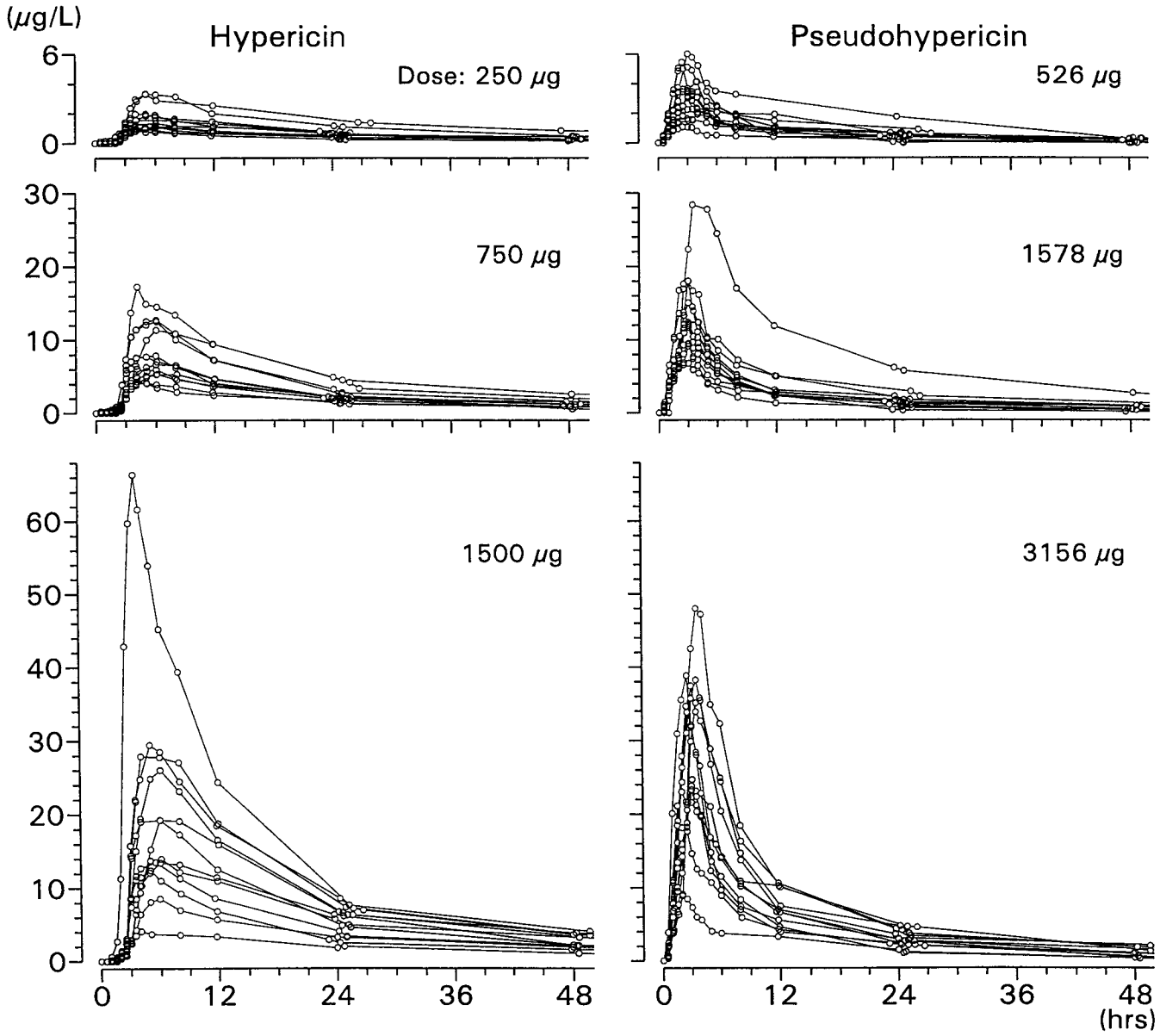

FIG. 2. Concentration in plasma-time curves for H and PH after three different oral single doses of *Hypericum* extract in 12 healthy volunteers.

dosing interval at steady state, which is equivalent to $AUC_{0-\infty}$ after a single dose. The volume of distribution at steady state (V_{SS}) and for oral treatment with respect to systemic availability F (V_{SS}/F) and the volume of distribution with reference to the central compartment (V_C, for intravenous treatment only) were computed from the fitted parameters. Total clearance (*CI*) (after oral dosing with respect to systemic availability, *CI*/*F*) was calculated by dividing the dose applied (one dose during steady-state treatment) by $AUC_{0-\infty}$. The mean concentration in plasma (C_{mean}) was calculated as $AUC_{0-12}/12$ h. The accumulation index [$R_{AC} = (1 - e^{-\beta \cdot \tau})^{-1}$] was applied to predict the maximal concentration in plasma during steady state from single-dose data ($C^{SS}_{max} = C_{max} \cdot R_{AC}$) (22).

Statistics. The Wilcoxon signed rank test was applied to analyze the differences between H and PH. Repeated measured analysis of variance was used to compare the $t_{1/2\beta}$ and t_{max} between different dose levels and to evaluate the pharmacokinetic linearity by comparing C_{max} and AUC values after recalculation to a dose of 100 μg of applied substance. Comparison of kinetic parameters between single-dose and multiple-dose treatments was done with factorial analysis of variance and post hoc test according to Fisher. Differences were considered significant at P of ≤ 0.05 at the 2α level. The influence of body weight was assessed by linear regression analyses and by comparing the CVs of AUC and C_{max} before and after standardization to body weight.

RESULTS

Single-dose pharmacokinetics. The concentration in plasma-time curves for H and PH after oral administration of three different single doses of *Hypericum* extract are illustrated in Fig. 2, and the kinetic data are compiled in Table 1. H was still measurable at 72 h after the lowest dose and at 120 h for the two higher doses; whereas PH was undetectable in most cases after 72 h. This longer residence of H in plasma compared with that of PH is reflected in a twice-as-long elimination half-life. Remarkably, there was a 2-h lag time before H appeared in the systemic circulation, whereas the lag time of PH (0.4 h) was significantly shorter ($P < 0.001$, $n = 36$) (Fig. 3).

The prolonged lag time of H was confirmed by oral administration of a solution of 750 μg of pure H. The resulting t_{lag} of 1.8 h was the same as the results found in this volunteer after

TABLE 1. Pharmacokinetic parameters of H and PH after single oral doses of *Hypericum* extract (one, three, or six coated tablets of LI 160) in healthy volunteers ($n = 12$)

Parameter	Median (range) for:					
	H			PH		
	250 μg	750 μg	1,500 μg	526 μg	1,578 μg	3,156 μg
t_{lag} (h)	2.1 (1.4–2.5)	1.9 (1.8–2.4)	1.9 (1.5–2.0)	0.5 (0.2–0.9)	0.4 (0.3–1.0)	0.4 (0.3–0.5)
C_{max} (μg/liter)	1.3 (0.9–3.3)	7.2 (4.1–17.3)	16.6 (4.1–66.3)	3.4 (1.1–7.1)	12.1 (6.8–28.4)	29.7 (8.9–48.0)
C_{max}/100-μg dose[a]	0.5 (0.3–1.3)	1.0 (0.6–2.3)	1.1 (0.3–4.4)	0.7 (0.2–1.3)	0.8 (0.4–1.8)	0.9 (0.3–1.5)
t_{max} (h)	5.5 (4.0–8.0)	6.0 (4.1–8.1)	5.7 (3.5–6.1)	3.0 (2.0–5.0)	3.0 (2.5–3.5)	3.0 (1.5–4.0)
C_{mean} (μg/liter)	0.9 (0.5–2.2)	4.4 (2.6–9.9)	9.4 (2.8–35.4)	1.9 (0.6–2.9)	5.8 (3.7–16.5)	14.0 (4.6–23.3)
$AUC_{0-\infty}$ ($h \cdot \mu g \cdot liter^{-1}$)	41.4 (17.5–120)	198 (127–452)	494 (139–826)	45.0 (17.2–98.2)	140 (87.1–481)	285 (89.7–498)
$AUC_{0-\infty}$/100-μg dose[a]	16.6 (7–48)	26.4 (16.9–60.3)	32.9 (9.3–55)	8.5 (3.3–18.7)	8.9 (5.5–30.5)	9.0 (2.8–15.8)
$t_{1/2k_a}$ (h)	1.4 (0.3–2.4)	0.6 (0.3–1.8)	0.8 (0.5–2.7)	0.9 (0.3–1.8)	1.3 (0.5–1.8)	1.4 (0.3–1.7)
$t_{1/2\alpha}$ (h)	1.9 (1.4–8.3)	6.0 (2.8–8.0)	5.8 (2.1–11.5)	1.2 (1.2–4.5)	1.4 (1.2–4.7)	1.5 (1.2–2.0)
$t_{1/2\beta}$ (h)	24.5 (14.7–57.8)	43.1 (28.2–57.8)	48.2 (22.9–57.8)	18.2 (13.9–27.9)	24.8 (13.9–69.3)	19.5 (13.9–41.9)
Cl/F (ml/min)	101 (34.7–238)	63.3 (27.7–98.3)	51.0 (30.3–180)	195 (89.2–511)	188 (54.7–302)	185 (106–586)
V_{SS}/F (liter)	111 (32.3–280)	69.6 (41.0–147)	73.3 (18.5–297)	117 (40.6–519)	61 (24.1–134)	50.0 (28.8–209)

[a] Data are recalculated to 100 μg of administered substance (either H or PH).

three coated tablets of LI 160. No PH was detectable after pure H.

The 6-fold increase in the H dose resulted in a 12-fold increase in C_{max} and $AUC_{0-\infty}$ (Table 1). This overproportional increase mainly resulted from the increment in the dose from 250- to 750-μg, whereas no substantial nonlinearity was demonstrated between 750- and 1,500-μg doses. The differences between the doses were statistically significant for the $AUC_{0-\infty}$ of H (P of 0.045) but only marginally significant for C_{max} (P of 0.058). For PH, this nonlinearity was less pronounced (Table 1). The terminal elimination half-life of H increased significantly with higher doses (P of 0.016), but this finding was not observed for PH.

Steady-state pharmacokinetics. Upon application of *Hypericum* extract containing 250 μg of H and 526 μg of PH three times a day (t.i.d.) (study part II), the steady-state level was reached after 6 to 7 days for H and after about 4 days for PH (Fig. 4). For H and PH, the kinetic parameters $t_{1/2\alpha}$, $t_{1/2\beta}$, CI/F, and V_{SS}/F were not significantly different from those in the single-dose study (Table 2). $AUC_{0-\infty}$ values for H and PH were similar to those after the same single doses. Estimation of peak levels in plasma for steady-state treatment on the basis of the C_{max} and accumulation index from the lowest single-dose (assuming a mean dosage interval of 8 h) underestimated the levels of H (6.4 μg/liter predicted versus 8.8 μg/liter observed) and overestimated the levels of PH (12.9 μg/liter predicted versus 8.5 μg/liter observed). The median trough levels were about two times higher for H than for PH and were characterized by a high intraindividual variation of factor 2.0 (range, 1.2 to 3.9) and 2.4 (1.4 to 5.3), respectively. Fluctuation between peak and trough levels was lower for H than for PH (29.7 versus 45.5%). In four volunteers, the concentration in plasma-time curve was remarkably flat, with little difference between peak and trough levels of H.

Dependency on body weight. No change in the population variance of C_{max} and AUC was observed after normalization to body weight. Linear regression analysis indicated an insignificant trend of decreasing AUC with increasing body weight for both naphthodianthrones (data not shown).

Intravenous pharmacokinetics. The kinetic parameters of H and PH after intravenous administration in two subjects are given in Table 3. The terminal elimination half-lives were similar to those of oral dosing (Table 1). H and PH were initially distributed into a small V_C of 4 to 5 liters, which is approximately the blood volume. The steady-state volume of distribution was compatible with the estimates (V_{SS}/F) obtained after oral dosage. The comparison of AUCs after intravenous and single oral dosing upon standardization to 100 μg of H or PH

TABLE 2. Pharmacokinetic parameters for H and PH during multiple dosing of *Hypericum* extract (one coated tablet of LI 160 t.i.d. for 14 days) in healthy volunteers ($n = 13$)

Parameter	Median (range) for:	
	H (250 μg t.i.d.)	PH (526 μg t.i.d.)
C^{SS}_{max} (μg/liter)[a]	8.8 (5.7–22.1)	8.5 (4.3–20.7)
C^{SS}_{min} (μg/liter)[b]	7.9 (3.4–13.6)	4.8 (1.1–10.1)
Fluctuation (%)[b]	29.7 (1.2–70.2)	45.5 (14.0–84.6)
$AUC_{0-\infty}$, ($h \cdot \mu g \cdot liter^{-1}$)	61.5 (39.6–152)	50.9 (30.7–108)
$t_{1/2k_a}$ (h)	2.0 (0.5–3.5)	1.7 (0.7–5.3)
$t_{1/2\alpha}$ (h)	2.0 (1.1–13.1)	1.9 (1.0–5.8)
$t_{1/2\beta}$ (h)	41.3 (30.1–71.4)	18.8 (13.9–46.2)
Cl/F (ml/min)	68.2 (27.4–105)	172 (81.3–286)
V_{SS}/F (liter)	162 (34.0–346)	63.0 (29.2–158)

[a] Day 14 of treatment.
[b] Days 7 to 14 of treatment.

TABLE 3. Pharmacokinetic parameters for H and PH after intravenous dosing of *Hypericum* extract in two subjects

Parameter	Result for[a]:			
	H (115 μg)		PH (38 μg)	
	Volunteer 1	Volunteer 2	Volunteer 1	Volunteer 2
C_{max} (μg/liter)	29.5	24.6	6.8	6.5
C_{24h} (μg/liter)	1.5	1.6	ND	ND
$AUC_{0-\infty}$ ($h \cdot \mu g \cdot liter^{-1}$)	205 (243)	194 (12.3)	19.1 (0.74)	11.9 (0.45)
$t_{1/2\alpha_1}$ (h)	0.77 (0.65)	0.45 (0.14)	0.15 (0.14)	0.25 (0.08)
$t_{1/2\alpha_2}$ (h)	3.8 (2.7)	4.1 (0.54)	0.79 (0.11)	1.1 (0.11)
$t_{1/2\beta}$ (h)	39.9 (149)	43.9 (12.2)	22.8 (2.8)	17.4 (3.9)
Cl (ml/min)	9.3 (11.1)	9.0 (1.28)	33.3 (0.76)	53.3 (2.15)
V_c (liter)	4.0 (0.20)	4.4 (0.12)	4.9 (0.41)	5.1 (0.24)
V_{SS} (liter)	18.5 (52)	20.9 (3.9)	44 (4.6)	34.5 (6.4)

[a] Asymptotic standard errors of estimated parameters are given in parentheses. The body weights were 81 kg for volunteer 1 and 66 kg for volunteer 2. ND, not detectable.

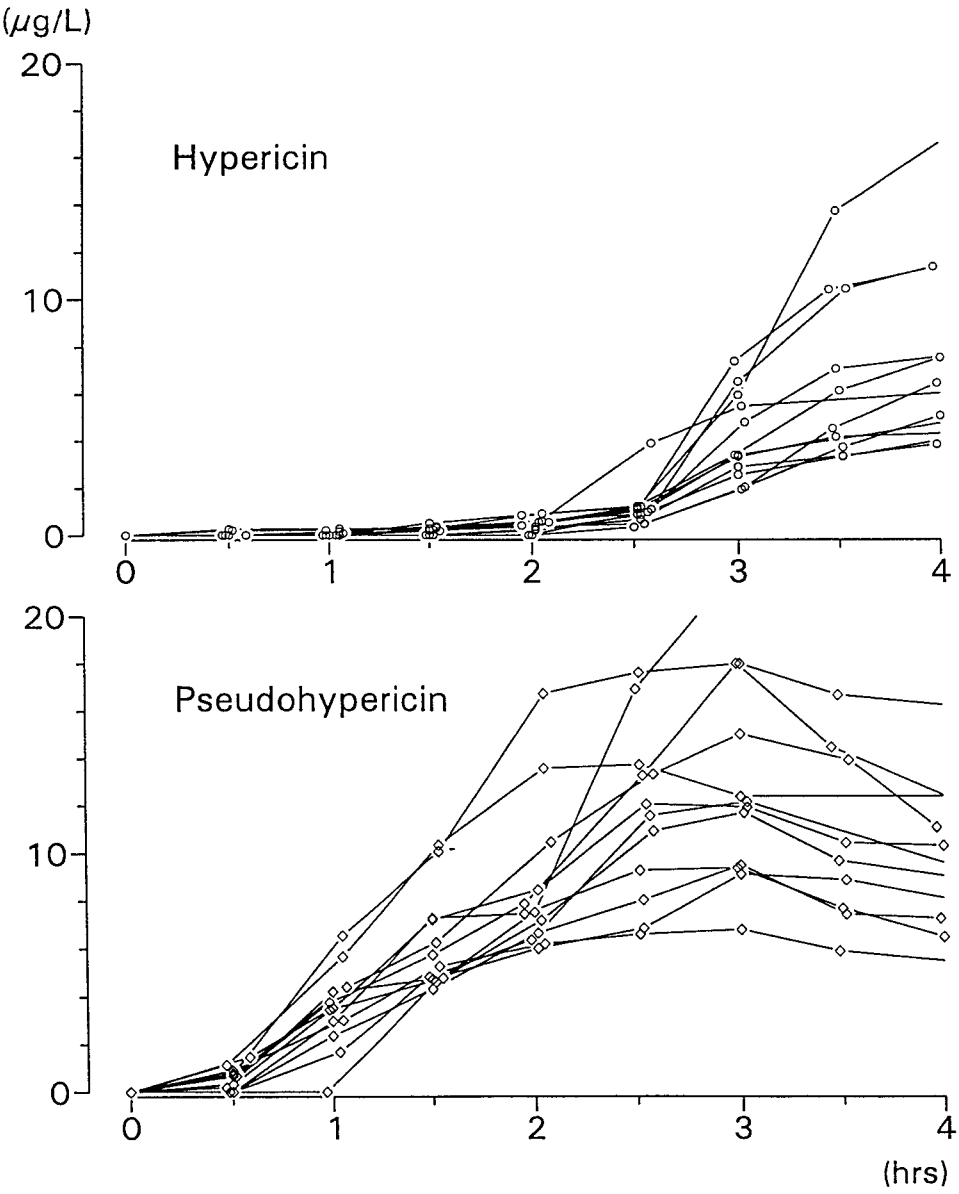

FIG. 3. Illustration of the prolonged lag time of H compared with that of PH (750- and 1,578-μg doses, respectively [Fig. 2]).

permits rough estimates of the systemic availability of 14% for H and 21% for PH.

Tolerability of medication. Single-dose and steady-state treatment was well tolerated. Headache and fatigue occurred sporadically, irrespective of dose, and only during those days when the subjects had to stay indoors to give repeated blood samples (every first day of single dosing and days 1 and 13 of steady state). No abnormality of any laboratory parameter and no skin reactions were observed, even after the highest single dose of 1,800 mg of *Hypericum* extract.

DISCUSSION

To date, few studies have investigated the pharmacokinetics of H and PH. Aside from our preliminary communication (25), kinetic data were available only for mice (19). In that study, the half-life of 38.5 h for H was surprisingly similar to our findings in man. In contrast to the lack of kinetic knowledge, the consumption of drugs containing H and PH is increasing. In 1994, the accounts of *Hypericum*-containing drugs showed 106 million daily doses given in Germany; compared with the number of doses reported in 1993, this was an increase of 31% (23).

Recently, the virucidal properties of the naphthodianthrones have become the subject of comprehensive research. Photochemical alteration of the virus capsid mediated by H was suggested to reduce reverse transcriptase activity and to prevent uncoating and subsequent reverse transcription of the virus genome within a target cell (7). Light enhanced the virucidal potency of H (14), and one proposed mechanism was lipid peroxidation by singlet oxygen which could be generated by H in the presence of light (18). Since the same mechanism may be involved in phototoxicity of H (24), antivirally acting concentrations may be toxic in humans. Concentrations of H or PH of 100 μg/ml were demonstrated to be virucidal in vitro (1), requiring an average dose of about 3 mg of H or 6 mg of PH,

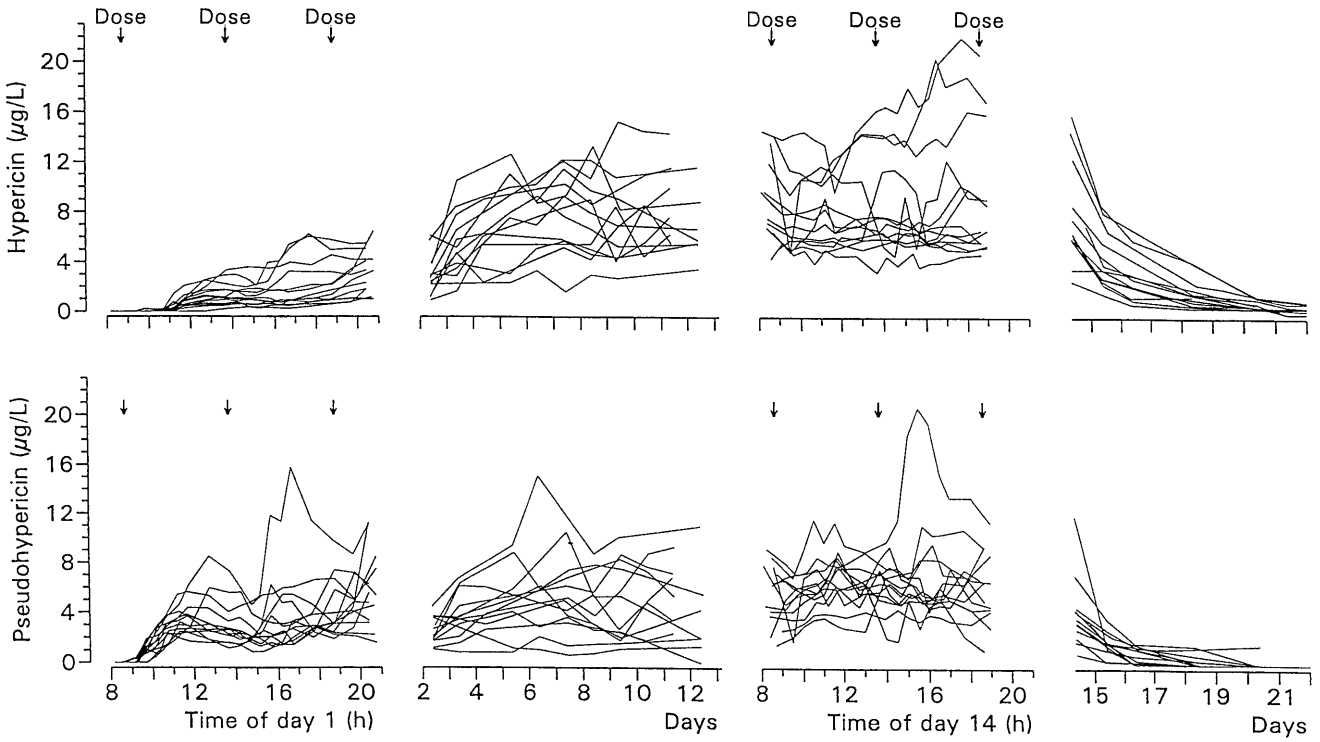

FIG. 4. Concentration in plasma-time curves for H and PH after multiple dosages of *Hypericum* extract (n = 13). From day 1 to 14 the dosing schedule was 250 μg of H and 526 μg of PH t.i.d. On days 2 to 13 and days 15 to 23, only the morning trough levels were determined.

whereas in single individuals much lower doses may be sufficient. In this study the highest peak concentration in plasma was 105 μg of total H and PH (66 μg of H plus 39 μg of PH) per liter. This concentration is within the range of being antivirally active in vitro, especially if one presumes that both H and PH are active. This concentration in plasma was achieved from a single dose of six coated tablets of LI 160, and up to 41 μg of total H and PH (20 μg of H plus 21 μg of PH) per liter resulted from long-term treatment with three coated tablets per day. No side effects, such as erythema of the skin, were detected, although all volunteers avoided prolonged exposure to direct sunlight. Even higher levels of H and PH in plasma (up to 98 μg/liter during chronic treatment) did not show a significant increase in dermal photosensitivity after irradiation with defined doses of UVA and UVB light (15).

A surprising observation was the prolonged lag time for H, ranging from 1.4 to 2.5 h, whereas the t_{lag} for PH, which is an ingredient of the same galenic preparation, was approximately 0.4 h. Since the administration of a solution of pure H confirmed this finding, a delayed liberation of H from the coated tablets was excluded. H is most likely absorbed at distal enteral sites.

With the exception of the first dose on day 1, a fluctuating concentration in plasma-time profile was not frequently observed during steady-state treatment. Since the multiple-dose $AUC_{0-\infty}$ was not significantly different from the single-dose $AUC_{0-\infty}$, autoinduction of metabolizing enzymes is not likely to provide an explanation of the flat concentration in plasma-time curves after 14 days of drug intake (Fig. 4). Variation of absorption is assumed as a source of pharmacokinetic variability after oral treatment, which is supported by the low systemic availability; food may interact with the absorption of H and PH because H demonstrated a high nonspecific affinity to proteins, detergents, and lipids (27). Cyclosporine has similar amphophilic properties; special galenic preparations, in combination with lipids, lead to formation of micelles (containing lipids, glucuronides, and cyclosporine) and result in an improved and more constant absorption (21). Additionally, H and PH absorption may be influenced by the PH value of the gastrointestinal system; this can be suspected from the chemical structure of H and PH. Another major source of variability may be intersubject differences of certain foreign-compound-metabolizing enzyme activities responsible for transformation of H and PH.

To extrapolate the H and PH dosage that is necessary for potential antiviral therapy, the question of kinetic linearity had to be addressed. After standardization to 100 μg of applied substance, the median AUC (and C_{max}) of H at 750- and 1,500-μg doses exceeded the values predicted from the 250-μg dose by factors of 1.5 and 2, respectively. The first step of dose elevation, from 250 to 750 μg of H, results in a nonlinear concentration response. Possibly, in the lowest dosing range, saturation of various binding sites is still taking place. However, to obtain conclusive results as to the nonlinearity of dose and kinetic response, more dose levels should be tested.

H and PH were not detectable in the urine or after incubation of urine with glucuronidase and sulfatase. From the chemical structure and molecular size (>500 Da), conjugation with glucuronic acid and subsequent excretion into bile seem likely. Metabolic formation of PH was not observed in the volunteer who received purified H only, nor was disposition kinetics of PH overlaid by PH formation from H after intravenous application of *Hypericum* extract.

Because of the observed variability in kinetics and the so-far-unknown upper therapeutic limit, monitoring of levels in plasma is recommended in clinical studies employing high

doses of H and PH. Further efforts are required to evaluate the metabolism of H and PH and to clarify the impact of food, age, and liver function on their pharmacokinetics.

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REFERENCES

1. **Andersen, D. O., N. D. Weber, S. G. Wood, B. G. Hughes, B. K. Murray, and J. A. North.** 1991. In vitro activity of selected anthraquinones and anthraquinone derivates. Antivir. Res. **16:**185–196.
2. **Araya, O. S., and E. J. H. Ford.** 1981. An investigation of the type of photosensitization caused by the ingestion of St. John's Wort (hypericum perforatum) by calves. J. Comp. Pathol. **91:**135–141.
3. **Barnard, D. L., J. H. Huffman, J. L. B. Morris, S. G. Wood, B. G. Hughes, and R. W. Sidwell.** 1992. Evaluation of the antiviral activity of anthraquinones, anthrones and anthraquinone derivatives against human cytomegalovirus. Antivir. Res. **17:**63–77.
4. **Brockmann, H. V., and D. Spitzner.** 1975. Die Konstitution des Pseudohypericins. Tetrahedron Lett. **24:**37–40.
5. **Carpenter, S., M. J. Fehr, G. A. Kraus, and J. W. Petrich.** 1994. Chemiluminescent activation of the antiviral activity of hypericin: a molecular flashlight. Proc. Natl. Acad. Sci. USA **91:**12273–12277.
6. **Conolly, K. J., and S. M. Hammer.** 1992. Antiretroviral therapy: strategies beyond single-agent reverse transcriptase inhibition. Antimicrob. Agents Chemother. **36:**509–520.
7. **Degar, S., A. M. Prince, D. Pascual, G. Lavie, B. Levin, Y. Mazur, D. Lavie, L. S. Ehrlich, C. Carter, and D. Meruelo.** 1992. Inactivation of the human immunodeficiency virus by hypericin: evidence for photochemical alterations of p24 and a block in uncoating. AIDS Res. Hum. Retroviruses **8:**1929–1936.
8. **Derendorf, H., and E. R. Garret.** 1987. Pharmakokinetik. Wissenschaftliche Verlags-Gesellschaft, Stuttgart.
9. **Diwu, Z.** 1995. Novel therapeutic and diagnostic applications of hypocrellins and hypericins. Photochem. Photobiol. **61:**529–539.
10. **Freytag, W. E.** 1984. Bestimmung von Hypericin und Nachweis von Pseudohypericin in Hypericum perforatum L. durch HPLC. Dtsch. Apoth. Ztg. **123:**2383–2386.
11. **Gabrielsson, J., and D. Weiner.** 1994. Pharmacokinetic/pharmacodynamic data analysis: concepts and applications. Swedish Pharmaceutical Press, Stockholm.
12. **Giese, A. C.** 1980. Hypericism. Photochem. Photobiol. Rev. **5:**229–255.
13. **Harrer, G., and H. Sommer.** 1994. Treatment of mild/moderate depressions with Hypericum Phytomed. **1:**3–8.
14. **Hudson, J. B., L. Harris, and G. H. Towers.** 1993. The importance of light in the anti-HIV effect of hypericin. Antiviral Res. **20:**173–8.
15. **Kerb, R., T. Reum, J. Brockmöller, S. Bauer, and I. Roots.** 1995. No clinically relevant photosensitization after single-dose and steady state iv treatment with hypericum extract in man. Eur. J. Clin. Pharmacol. **49:**A156.
16. **Lavie, G., Y. Mazur, D. Lavie, A. M. Prince, D. Pascual, L. Liebes, B. Levin, and D. Meruelo.** 1995. Hypericin as an inactivator of infectious viruses in blood components. Transfusion **35:**392–400.
17. **Lavie, G., F. Valentine, B. Levin, Y. Mazur, G. Gallo, D. Lavie, D. Weiner, and D. Meruelo.** 1989. Studies of the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin. Proc. Natl. Acad. Sci. USA **86:**5963–5967.
18. **Lenard, J., A. Rabson, and R. Vanderoef.** 1993. Photodynamic inactivation of infectivity of human immunodificiency virus and other enveloped viruses using hypericin and rose bengal: inhibition of fusion and syncytia formation. Proc. Natl. Acad. Sci. USA **90:**158–162.
19. **Liebes, L., Y. Mazur, D. Freeman, D. Lavie, G. Lavie, N. Kudler, S. Mendoza, B. Levin, H. Hochster, and D. Meruelo.** 1991. A method for the quantification of hypericin, an antiviral agent, in biological fluids by high-performance liquid chromatography. Anal. Biochem. **195:**77–85.
20. **Meruelo, D., G. Lavie, and D. Lavie.** 1988. Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. Proc. Natl. Acad. Sci. USA **85:**5230–5234.
21. **Mueller, E. A., J. M. Kovarik, J. B. Van Bree, A. E. Lison, and K. Kutz.** 1994. Pharmacokinetics and tolerability of a microemulsion formulation of cyclosporine in renal allograft recipients—a concentration-controlled comparison with the commercial formulation. Transplantation **57:**1178–1182.
22. **Rowland, M., and T. N. Tozer.** 1989. Clinical pharmacokinetics: concepts and applications, 2nd ed. Lea & Febiger, Philadelphia.
23. **Schwabe, U., and D. Paffrath.** 1995. Arzneiverordnungs-Report '95: aktuelle Daten, Kosten, Trends und Kommentare, p. 375. Gustav Fischer Verlag, Stuttgart.
24. **Senthil, V., J. W. Longworth, C. A. Ghiron, and L. I. Grossweiner.** 1992. Photosensitization of aqueous model systems by hypericin. Biochim. Biophys. Acta **1115:**192–200.
25. **Staffeldt, B., R. Kerb, J. Brockmöller, M. Ploch, and I. Roots.** 1994. Pharmacokinetics of hypericin and pseudohypericin after oral intake of the hypericum perforatum extract LI 160 in healthy volunteers. J. Geriatr. Psychiatry Neurol. **7**(Suppl. 1)**:**47–53.
26. **Venning, G. R., and M. S. Scott.** 1992. New drug treatments for HIV infection and AIDS: a review following the 7th International Conference on AIDS, Florence, 16 to 21 June 1991. Br. J. Clin. Pharmacol. **33:**349–356.
27. **Weber, N. D., B. K. Murray, J. A. North, and S. G. Wood.** 1994. The antiviral agent hypericin has in vitro activity against HSV-1 through non-specific association with viral and cellular membranes. Antiviral Chem. Chemother. **5:**83–90.